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Effect of Variation of Process Parameters on Stability of Colloidal Metal-Complexes

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ABSTRACT

In this manuscript the stabilty of colloidal metal complexes is studied in detail to resolve the stability issues of the iron complex formulations. It was observed that the method of preparation of the iron complexes determines its overall stability as well as the morphology of the metal-polysaccharide nanoparticles. Some alterations to existing methodology has been proposed in this mauscript that will help stabilize the metal-polysaccharide colloidal complexes.

Keywords: Colloid, Metal-complexes, Stability, Polysaccharide, Iron, Gluconate, Sodium Ferric Gluconate, Ferric Gluconate, Sodium Gluconate, Iron Complex, Iron Nanoparticles, GPC

INTRODUCTION

Intravenous iron preparations are nothing but colloids consisting of iron carbohydrate nanoparticles. At the center of every particle, an iron-oxyhydroxide core is present. A shell of carbohydrate molecule surrounds the core and thus stabilizes the iron core, retards the bioactive iron release and also sustains the resulting particles in the colloidal solution. Iron preparations used in the clinic comprise of various types e.g. iron dextran, iron sucrose and iron gluconate, which have a common core, but vary from each other because of their size and identity of the carbohydrate molecule that surrounds them. This leads to pharmacologic as well as biologic differences, such as pharmacokinetic profile, iron release rate and the maximum tolerated dose. Iron gluconate and iron sucrose have more favorable safety profiles than iron dextran.

The preparation of these complexes consists of combination of a ferric salt solution along with a weak alkali either of sodium carbonate, sodium bicarbonate, lithium carbonate, potassium carbonate, potassium bicarbonate, ammonium carbonate or ammonium bicarbonate to form ferric oxyhydroxide. The preparation of ferric oxyhydroxide is the key step and a colloidal gel is formed. As described below, the chloride ions and carbon dioxide etc. are the key by-products of this neutralization reaction of a weak base with a weak acidic solution of ferric chloride

The following scheme is proposed for the formation of ferric oxyhydroxide

$$FeCl_3 + H_2O \rightarrow Fe^{3+} + 3Cl^-$$

$$Fe^{3+} + H_2O \rightleftarrows Fe(OH)_2^{+} + H^+$$

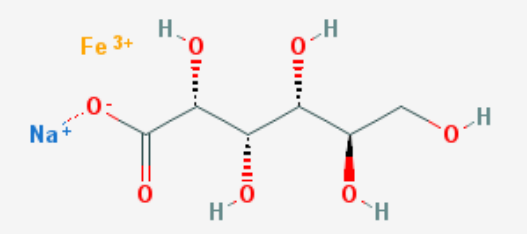
$$Fe(OH)_2^{+} + H_2O \rightleftarrows Fe(OH)_2^{+} + H^+$$

$Fe^{3+} + CO_3^{-2}$ (if sodium carbonate used in process) $+H_2O \rightarrow Fe(OH)^{+2} + HCO_3^{-}1$, Cl^{-1} (from ferric chloride) + Na^{+1} (if Na_2CO_3 or $NaHCo_3$ used in process) → NaCl, Cl^{-1} (from ferric chloride) + Li^{+1} (if lithium carbonate used in process) → LiCl, Cl^{-1} (from ferric chloride) + K^{+1} (if K_2CO_3/$KHCO_3$ used in process) → KCl, Cl^{-1} (from ferric chloride) + NH_4^{+1} (is $(NH_4)_2CO_3$ or NH_4HCO_3 used in process) → NH_4Cl, $Fe(OH)^{+2} + HCO_3^{-1} + H_2O \rightarrow Fe(OH)_2^{+1} + CO_2\uparrow$ (in solution)

$$(Poly)Fe(OH)_2^{+1} + CO_3^{-2} + H_2O + Cl^{-1} \rightarrow (poly)\ FeOOH\ (colloidal\ gel) + Cl^{-1} + Co_2\uparrow$$

Certain low molecular mass neutral carbohydrates, such as sucrose or fructose present multiple hydroxyl groups in a suitable array to chelate iron, although the binding is inherently weak in neutral aqueous solution. The chelation process to iron is usually enhanced at high pH in case of neutral carbohydrates, since the hydroxyl groups might get deprotonated, resulting in a negative charge, and thus binds more strongly with the cationic iron ion.

At neutral pH, inherently anionic carbohydrates like gluconate or partially oxidized polysaccharides are found to be better as nanoparticle stabilizers. The electronic environment of the iron is maintained with a polyneuclear iron oxyhydroxide core and the structure is stabilized by the interacting carbohydrate. Iron oxyhydroxide in the form of β-FeOOH mineral polymorph (called akaganeite) comprises the core. The preparation of sodium ferric gluconate consists of reaction of a ferric salt solution along with a weak alkali (alkaline earth metals and ammonium salts group, such as sodium bicarbonate, sodium carbonate, lithium carbonate, ammonium carbonate, potassium carbonate, potassium bicarbonate, ammonium bicarbonate and their mixtures are preferred), to obtain ferric oxyhydroxide. Combining ferric oxyhydroxide with sodium gluconate yields sodium ferric gluconate complex ($C_6H_{11}FeNaO_7^{+3}$) molecular weight (273.982109 g/mol) (Figure 1) [1-40].



Hydrogen bond donor count	5
Hydrogen bond acceptor count	7
Rotatable bond count	5
Exact mass	273.975184 g/mol
monoisotopic mass	273.975184 g/mol
Topological polar surface area	141 A^2
Heavy atom count	15
Formal charge	3
Complexity	176
Isotope atom count	4
Undefined bond stereo center count	3

Figure 1: sodium ferric gluconate complex

EXPERIMENTAL

Preparation of sodium ferric gluconate (Table 1)

Example 1

Ferric chloride hexahydrate (100 g) and water (1000 ml) were stirred at room temperature for around 30 min and cooled to 10°C. Sodium carbonate solution (58 g) in water (200 ml) was added to the above mixture at 10°C in 6-7 h. The reaction mixture was then stirred for 10 min and the solid thus obtained was collected via filtration and was given water wash (1000 ml) to get Ferric oxyhydroxide. A mixture of sodium gluconate (40 g) in water (200 ml) was heated to 95°C. The above obtained ferric oxyhydroxide slurry in water was added followed by sodium hydroxide (30 ml) to attain basic pH. The obtained mixture was maintained at 90°C for 2-4 h and cooled to an ambient temperature and pH was adjusted to 9-10 to get the sodium ferric gluconate solution. Ethanol was added to the above solution to isolate the solid. The crystalline solid thus obtained was collected by filtration and washed with ethanol. The obtained solids were vacuum dried at 40 ± 5°C to get sodium ferric gluconate solid, which is hygroscopic in nature.

Example 2

Ferric chloride hexahydrate (100 g) and water (1000 ml) were stirred at room temperature for around 30 min and cooled to 10°C. Sodium carbonate solution (58 g) in water (200 ml) was added to the above mixture at 10°C in 6-7 h. The reaction mixture was then stirred for 10 min and the solid thus obtained was collected via filtration and was given water wash (1000 ml) to get ferric oxyhydroxide. A mixture of sodium gluconate (40 g) in water (200 ml) was heated to 95°C. The above obtained ferric oxyhydroxide slurry in water was added followed by sodium hydroxide (30 ml) to attain basic pH. The obtained mixture was maintained at 90°C for 2-4 h and cooled to an ambient temperature and pH was adjusted to 9-10 to get the sodium ferric gluconate solution & solid was isolated by using methanol. The resulting crystalline solid was collected by filtration and washed with methanol. The obtained solid was vacuum dried at 40 ± 5°C to form solid sodium ferric gluconate, which is hygroscopic in nature.

Example 3

Ferric chloride hexahydrate (100 g) and water (1000 ml) were stirred at room temperature for around 30 min and cooled to 10°C. Sodium carbonate solution (58 g) in water (200 ml) was added to the above mixture at 10°C in 6-7 h. The reaction mixture was then stirred for 10 min and the solid thus obtained was collected via filtration and was given water wash (1000 ml) to get ferric oxyhydroxide. A mixture of sodium gluconate (40 g) in water (200 ml) was heated to 95°C. The above obtained ferric oxyhydroxide slurry in water was added followed by sodium hydroxide (30 ml) to attain basic pH. The obtained mixture was maintained at 90°C for 2-4 h and cooled to an ambient temperature and pH was adjusted to 9-10 to get the sodium ferric gluconate solution & solid was isolated by using Isopropyl alcohol. The resulting crystalline solid was collected by filtration and washed with Isopropyl alcohol. The obtained solid was dried under vacuum at 40 ± 5°C to get solid sodium ferric gluconate, which is hygroscopic in nature.

Example 4

Ferric chloride hexahydrate (100 g) and water (1000 ml) were stirred at room temperature for around 30 min and cooled to 10°C. Sodium carbonate solution (58 g) in water (200 ml) was added to the above mixture at 10°C in 6-7 h. The reaction mixture was then stirred for 10 min and the solid thus obtained was collected via filtration and was given water wash (1000 ml) to get ferric oxyhydroxide. A mixture of sodium gluconate (40 g) in water (200 ml) was heated to 95ºC.

The above obtained ferric oxyhydroxide slurry in water was added followed by sodium hydroxide (30 ml) to attain basic pH. The obtained mixture was maintained at 90°C for 2-4 h and cooled to an ambient temperature and pH was adjusted to 9-10 to get the sodium ferric gluconate solution. Solution is concentrated using diafiltration process (1 KDa-100 KDa) till the specific conductivity was found to be below 3 ms/cm. Under reduced pressure, the solution was then concentrated to get the required quantity basing on iron content required in solution phase.

Example 5

Ferric chloride hexahydrate (100 g) and water (1000 ml) were stirred at room temperature for around 30 min and cooled to 10°C. Sodium carbonate solution (58 g) in water (200 ml) was added to the above mixture at 10°C in 6-7 h. The reaction mixture was then stirred for 10 min and the solid thus obtained was collected via filtration and was given water wash (1000 ml) to get ferric oxyhydroxide. A mixture of sodium gluconate (40 g) in water (200 ml) was heated to 95°C. The above obtained ferric oxyhydroxide slurry in water was added followed by sodium hydroxide (30 ml) to attain basic pH. The obtained mixture was maintained at 90°C for 2-4 h and cooled to an ambient temperature and pH was adjusted to 9-10 to get the sodium ferric gluconate solution & the solution was concentrated using membrane process (diafiltration) until the specific conductivity was found to be below 10 ms/cm. Sample is tested for sodium and gluconate contents, if required more sodium gluconate was added. Solid was isolated by adding ethanol to above solution. The resulting crystalline solid was collected by filtration and washed with ethanol. The obtained solids were dried under vacuum at 40 ± 5°C to get solid sodium ferric gluconate, which is hygroscopic in nature

Example including a hydroxide kind of base is described below

To a ferric chloride hexahydrate solution (6.7 g) in water (100 ml), sodium hydroxide solution (2.9 g in 50 ml water) was added gradually over 10 min times, with continuous stirring. The colloidal ferric hydroxide thus obtained was washed with water (5 × 100 ml) multiple times, to get rid of the chloride salt (silver nitrate solution test was also done). Then 2.7 g of sodium gluconate (solid) was added to the ferric hydroxide suspended in 250 ml of water at about 25°C with continuous stirring over 5 min time. Post this, the reaction mass was heated to 70-80°C for over 4 h. Color change of the reaction mixture to dark brown color was not observed, and suspended solids were also seen. This proved that the reaction was not successful.

Table 1: Sodium ferric gluconate conductivity and Total Dissolved Solids (TDS)

Details	Conductivity	TDS
Reaction mass during formation of β-FeOOH reaction	36.7 ms/ppt	24589 mg/l
Sodium ferric gluconate reaction mass Without filtration of β-FeOOH	71.8 ms/ppt	48106 mg/l
Solution of sodium ferric gluconate reaction mass without filtration & washing of β-FeOOH and after purification using tangential flow filtration	6.58 ms/ppt	4408.6 mg/l
Solution of sodium ferric gluconate reaction mass Without filtration & washing of β-FeOOH and after purification	45.9 ms/ppt	30753 mg/l
Solution of sodium ferric gluconate reaction mass with filtration & washing of β-FeOOH and after purification	31.5 ms/ppt	21105 mg/l

RESULT AND DISCUSSION

The complex structures of iron nanoparticles and polysaccharides have been talked about from a long time since iron dextran complexes [8,9] as well as iron polymaltosate complexes [10-12] (partially hydrolyzed and oxidized starch was called as polymaltosate commercially) are actively used in treating anemia since ages. In many cases, the core of the nanoparticles was identified as iron oxyhydroxide. X-ray diffraction data obtained were seen to be matching with the akaganeite β-FeOOH polymorph (polymorphs are those solids that have the same chemical profile but different crystal structures) [13-18]. Ultraviolet-visible (UV) absorption spectroscopy [5,17-19] and Mossbauer spectroscopy [13-16,20], confirmed the presence of Fe (III) ions that have octahedrally coordinated high spin. Extended X-ray Absorption Fine Structure (EXAFS) studies [14] confirmed the coordination of iron by 6 oxygen atoms, at a Fe-O distance of 1.95 A˚ and disordered kind of shell of iron ions were seen to be located at a distance of about 3.05 A ˚. The broadening of X-ray diffraction peaks of about 1-5 nm in diameter [9,14,17] clarified the iron oxyhydroxide crystallite dimensions estimation. The core size (can also be larger than the crystalline portion size) ranges from spherical shape (3 nm diameter), [9] to ellipsoidal shapes (sized upto 5.34 nm), as seen in electron microscopy [21]. The hydroxyl and/or carboxyl groups (generated by partial oxidation of the polysaccharide) of the carbohydrate units of the polysaccharide interact with the mineral core to stabilize it. The complete particle size depends on the polysaccharide`s nature and also on the mode of complex formation. One of the older commercialized iron–dextran complexes reported an overall diameter between 12-13 nm [9]. However, j-carrageenan reported 25-50 nm aggregates of 10-20 nm particles of iron complexes. [17,18]. Based on the elution position in gel permeation chromatography (GPC) chromatograms, the apparent complex molecular mass might range from 72 kDa (InFed) to 90 kDa (Imferon) to 265 kDa (Dexferrum). By studying soluble di or mono-nuclear iron complexes, structures of iron complexes having low molecular mass carbohydrates were determined. Carbohydrates having structures that allow a minimum of three hydroxyl groups to freely interact with a single iron ion, all at once, form weakly stable mononuclear complexes with them. Taking an example of sucrose or fructose, they weakly bind iron in neutral pH. There is a dearth of information when it comes to complexes that have polyneuclear iron species complexed with monosaccharaides or disaccharides. After studying ferric fructose systems, it was seen that fructose was primarily used to coat iron-containing particles in neutral pH. Once the pH is raised, the polyneuclear iron was broken down to the mononuclear ferric fructose complexes, due to the tetra-deprotonated sugar moiety that gets formed in the process. It was also found that gluconate (and its carboxyl group), was an effective source in breaking the particles down [5]. Previous studies on iron sucrose, suggested that a polyneuclear iron was complexed with sucrose, the mineral core being a 2-line ferrihydrite [22] instead of an iron oxyhydroxide.

The known methods used for preparation of iron (III) gluconate complex have several disadvantages. One of the main issues that is observed in all the methods of preparation of iron (III) gluconate complex, for e.g. sodium iron (III) gluconate complex, is the resultant chloride content separation that is formed from the iron chloride, especially the counter ion (formed from the iron oxyhydroxide) separation. This anion content is not desirable physiologically. In previously described methods, the separation of the chloride content is done from the slushy of iron (III) oxyhydroxide.

However, it is common knowledge regarding the difficulty of filtering freshly precipitated colloidal iron oxyhydroxide when compared to aged iron oxyhydroxide. Even though the latter is easier to filter, it cannot find use in the syntheses of physiologically active sodium iron (III) gluconate complex. Thus, the iron oxyhydroxide should be sloshed multiple times to decant the remaining solution. However, this method is not only expensive but also technically impractical.

As described herein, the process of production of solid sodium iron (III) gluconate, involves freeze-drying of the complex. Since all ions & salts will stay in solid obtained after freeze drying thus process usually gets complicated & removal of the other ions along with chloride become necessary step before the freeze-drying process. Freeze-drying is quite a time consuming procedure and it consumes a lot of energy. Other hand the separation of the chloride content from the freshly precipitated colloidal iron oxyhydroxide may not be necessary. Simple precipitation using an organic solvent can also yield solid iron (III) gluconate complex and hence the sodium iron (III) gluconate complex if made in accordance with the present invention, will not contain any undesired additives or carrier materials.

CONCLUSION

Sodium ferric gluconate can be prepared & purified in various ways, each method will end up in different ion concentrations depending upon conductivity, ions & total dissolved solids remained in the process. There might arise a few questions regarding the analysis of polyneuclear iron carbohydrate complexes like: (1) The polymorphic form of the iron mineral, its composition and the degree of its crystallinity. (2) The shape and size of the obtained nanoparticles. (3) The nanoparticle stability profile. (4) The exact location where the saccharide component is present inside the nanoparticle. (5) The iron to saccharide molar ratio. (6) The mode by which the iron and saccharide components are bound. This includes the extent to which saccharide deprotonation occurs in the complex.

The methods of preparation can define the stability of Sodium ferric gluconate colloidal products because colloids dispersed in water usually carry an electrical charge due to: (1) Surface group ionization: controlled by the pH of the dispersion medium. (2) Differential solubility of ions: e.g. Crystals are partially soluble in water and dissolved ions would preferably leave a negatively charged surface. (3) Isomorphous replacement: e.g. in kaolinite, Si^{4+} is replaced by Al^{3+} to give negative charges. (4) Charged crystal surface: Fracturing crystals can reveal surfaces with differing properties. (5) Specific ion adsorption: Surfactant ions may be specifically adsorbed. Therefore it is the method of preparation that determines the stability and morphology of metal-polysaccharide complexes.

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